D96W +G263Q +L264A +I265T +G266D +T267A +L269N +270AGGFS
G91A +D96W +E99K +G263Q +L264A +I265T +G266D +T267A +L269N +270AGGFS

8. A lipolytic enzyme which is a variant of a parent lipolytic enzyme having an alcohol binding site having a glycerol part with an sn2 position, which variant:

a) comprises an alteration which is an insertion, a deletion or a substitution of an amino acid residue, at a position which in a three-dimensional structure of the parent lipolytic enzyme and a substrate is within 10 Å of the C atom at the sn2 position of the glycerol part of a substrate triglyceride, and

b) has an altered activity on an ester bond in the substrate.

9. A lipolytic enzyme which is a variant of a parent lipolytic enzyme having a lid, which variant:

a) comprises an alteration which is an insertion, a deletion or a substitution of an amino acid residue in the lid,

b) has an altered activity on an ester bond in the substrate.

10. A lipolytic enzyme which is a variant of a parent lipolytic enzyme having an active site comprising an active His residue, which variant:

a) comprises an alteration which is an insertion, a deletion or a substitution of at least one amino acid residue at the C-terminal side of the active His residue,

b) has an altered activity on an ester bond in a substrate.

11. A lipolytic enzyme which is a variant of a parent lipolytic enzyme, which variant:

a) comprises an alteration which is an insertion, a deletion or a substitution of at least one amino acid within 10 amino acid residues of the C-terminal,

b) has an altered activity on an ester bond in a substrate.

12. A lipolytic enzyme which:

a) is a polypeptide having an amino acid sequence which has at least 80 % homology with a reference lipolytic enzyme of the *Humicola* family or the *Zygomycetes* family;

b) compared to said reference lipolytic enzyme comprises an amino acid alteration which is:

wherein the lipolytic enzyme preferably has phospholipase and/or digalactosyl diglyceride activity.

25. The process of claim 23 which further comprises adding to the dough an endo-amylase and/or a phospholipid.

26. The process of claim 25 wherein the endo-amylase is from *Bacillus*, and is preferably a maltogenic amylase from *B. stearothermophilus*,

27. A process for reducing the content of phospholipid in an edible oil, comprising treating the oil with the lipolytic enzyme of claim 4 which has phospholipase activity so as to hydrolyze a major part of the phospholipid, and separating an aqueous phase containing the hydrolyzed phospholipid from the oil.

28. A process for improving the filterability of an aqueous solution or slurry of carbohydrate origin which contains phospholipid, which process comprises treating the solution or slurry with the lipolytic enzyme of claim 4 which has phospholipase activity, wherein the solution or slurry preferably contains a starch hydrolysate, particularly a wheat starch hydrolysate.

29. A detergent composition comprising a surfactant and the lipolytic enzyme of any of claims 1-19, wherein the lipolytic enzyme preferably has a specificity for long-chain fatty acids corresponding to a ratio of SLU to LU above 3.

30. A method of enhancing the flavor of a food product containing milk fat, comprising treating the food product with the lipolytic enzyme of any of claims 1-19 so as to release free fatty acids, wherein the lipolytic enzyme preferably has a specificity for short-chain fatty acids corresponding to a ratio of SLU to LU below 0.5, more preferably below 0.2, e.g. below 0.1.

31. A method of producing a lipolytic enzyme variant comprising:

a) selecting a substrate and an ester bond of interest,

b) selecting a parent lipolytic enzyme having an alcohol binding site having a glycerol part with an sn2 position,

c) in the parent lipolytic enzyme selecting at least one amino acid residue which comprises at least one atom within 10 Å of the C atom at the sn2 posi-

tion of the glycerol part of a substrate triglyceride in a three-dimensional structure of the parent lipolytic enzyme and the substrate,

d) making alterations each of which is an insertion, a deletion or a substitution of the amino acid residue,

e) optionally, making alterations each of which is an insertion, a deletion or a substitution of an amino acid residue at one or more positions other than b),

f) preparing the variant resulting from steps b-d,

g) testing the activity of the variant on the selected ester bond,

h) selecting a variant having an altered activity on the selected ester bond, and

i) producing the selected variant.

32. A method of producing a lipolytic enzyme variant comprising:

a) selecting a substrate and an ester bond of interest,

b) selecting a parent lipolytic enzyme having a structure comprising a catalytic triad consisting of an active Ser, an active Asp and an active His residue,

c) in the parent lipolytic enzyme selecting at least one amino acid residue comprising at least one atom belonging to a set E defined by the following steps:

i) aligning the structure of the lipolytic enzyme with *Rhizomucor miehei* lipase structure 4TGL comprising a catalytic triad and an inhibitor phosphorus atom (4TGL-inhP), so as to minimize the sum of squares of deviation between atoms of the catalytic triads of the two structures,

ii) defining a set A consisting of atoms of the lipolytic enzyme inside a sphere of radius 18 Å with center at 4TGL-inhP,

iii) forming a first plane defined by 4TGL-inhP, the Cα atom of the active Ser residue of the parent lipolytic enzyme, and the Cα atom of the active Asp residue of the parent lipolytic enzyme and defining a set B as a subset of set A consisting of atoms on the same side of the first plane as the Cα atom of the active His residue of the parent lipolytic enzyme,

iv) forming a second plane defined by 4TGL-inhP, the Cα atom of the active Ser residue of the parent lipolytic enzyme, and the Cα

atom of the active His residue of the parent lipolytic enzyme and defining a set C as a subset of set A consisting of atoms on the opposite side of the second plane from the C alpha atom of the active Asp residue of the parent lipolytic enzyme,

v) forming a set D consisting of atoms belonging to the union of sets B and C, and having a solvent accessibility of 15 or higher, and

vi) forming set E consisting of amino acid residues in the structure which comprise an atom belonging to set D or an atom belonging to the union of sets B and C and located less than 3.5 Å from an atom belonging to set D,

d) making alterations each of which is an insertion, a deletion or a substitution of the selected amino acid residues,

e) optionally, making alterations each of which is an insertion, a deletion or a substitution of an amino acid residue at one or more positions other than d),

f) preparing the variant resulting from steps d) - f), and

g) testing the activity of the variant on the selected ester bond,

h) selecting a variant having an altered activity on the selected ester bond, and

i) producing the selected variant.

33. A method of producing a lipolytic enzyme variant comprising:

a) selecting a substrate and an ester bond of interest,

b) selecting a parent lipolytic enzyme having an active site comprising an active His residue,

c) in the amino acid sequence of the parent lipolytic enzyme selecting at least one amino acid residue at the C-terminal side of the active His residue,

d) making alterations each of which is an insertion, a deletion or a substitution of the amino acid residue,

e) optionally, making alterations each of which is an insertion, a deletion or a substitution of an amino acid residue at one or more positions other than b),

f) preparing the variant resulting from steps b-d,

g) testing the activity of the variant on the selected ester bond,

h) selecting a variant having an altered activity on the selected ester bond, and

i) producing the selected variant.

34. A method of producing a lipolytic enzyme variant comprising:

a) selecting a substrate and an ester bond of interest,

b) selecting a parent lipolytic enzyme,

c) selecting at least one amino acid residue among 10 amino acid residues at the C-terminal,

d) making alterations each of which is an insertion, a deletion or a substitution of the selected amino acid residues,

e) optionally, making alterations each of which is an insertion, a deletion or a substitution of an amino acid residue at one or more positions other than c),

f) preparing the variant resulting from steps c) - e),

g) testing the activity of the variant on the selected ester bond,

h) selecting a variant having an altered activity on the selected ester bond, and

i) producing the selected variant.

35. A method of producing a lipolytic enzyme variant comprising:

a) selecting a substrate and an ester bond of interest,

b) selecting a parent lipolytic enzyme having a lid,

c) selecting at least one amino acid residue in the lid,

d) making alterations each of which is an insertion, a deletion or a substitution of the selected amino acid residues,

e) optionally, making alterations each of which is an insertion, a deletion or a substitution of an amino acid residue at one or more positions other than c),

f) preparing the variant resulting from steps c) - e),

g) testing the activity of the variant on the selected ester bond,

h) selecting a variant having an altered activity on the selected ester bond, and

i) producing the selected variant.

36. A method of producing a lipolytic enzyme variant comprising:

a) selecting a substrate and an ester bond of interest,

b) selecting a parent lipolytic enzyme from the *Humicola* family or the *Zygomycetes* family,

c) selecting at least one amino acid residue corresponding to any of amino acids 20-25, 56-64, 81-85 and 255-269 in the *Humicola lanuginosa* lipase

d) making alterations each of which is an insertion, a deletion or a substitution of the amino acid residue,

e) optionally, making alterations each of which is an insertion, a deletion or a substitution of an amino acid residue at one or more positions other than c),

f) preparing the variant resulting from steps b-e,

g) testing the activity of the variant on the ester bond in the substrate, and

h) selecting a variant having an altered activity on the ester bond.

37. The method of any of claims 31-36 wherein the altered activity is a lower activity towards a C_4-C_8 acyl bond in a triglyceride, or a lower ratio of activity towards a C_4-C_8 acyl bond in a triglyceride and a C_{16}-C_{20} acyl bond in a triglyceride.

38. The method of claim 37 wherein the parent lipolytic enzyme belongs to the *Humicola* family or the *Zygomycetes* family, preferably the lipase of *Humicola lanuginosa* strain DSM 4109, and the selected amino acid residues comprise an amino acid corresponding to Y21, E56, D57, V60, G61, D62, R81, S83, R84, L259, Y261 or G266 in the *Humicola lanuginosa* lipase.

39. The method of any of claims 31-36 wherein the altered activity is a higher hydrolytic activity on a digalactosyl-diglyceride.

40. The method of claim 39 wherein the parent lipolytic enzyme belongs to the *Humicola* family or the *Zygomycetes* family, preferably the lipase of *Humicola lanuginosa* strain DSM 4109, and the selected amino acid residues comprise an amino acid corresponding to 21, 23, 26, 57, 62, 81, 83, 84, 85, 266, 267 or 269 in the *Humicola lanuginosa* lipase.